

IT IS CLAIMED:

1. A method of detecting each or any of a plurality of known, selected nucleotide target sequences, comprising:

- 5 (a) mixing the target sequences with (i) a set of forward universal e-tag primers, each member of the set containing (ia) a target sequence that is complementary to one of the known selected target sequences, and (ib) an extension sequence which is unique to the target sequence of that member, (ii) one or more reverse primers that are complementary to said target sequences,  
10 and (iii) enzyme and nucleotide components of a primer extension reaction, to form a target-sequence reaction mixture;  
(b) reacting the reaction mixture under primer extension reaction conditions, to form extended target sequences,  
(c) reacting, under hybridization conditions, the extended target  
15 sequences with a set of electrophoretic tag (e-tag) probes, each member of the tag probe set having (i) an oligonucleotide portion that is complementary to one of said extension sequences, (ii) an electrophoretic probe having an electrophoretic mobility that is unique to a given extension sequence, and (iii) a linker joining the oligonucleotide portion and the electrophoretic probe, said linker  
20 being cleavable under selected conditions when the oligonucleotide portion of said probe is bound to a complementary target extension sequence,  
(d) treating the target sequences under said selected conditions, to release an e-tag reporter from each e-tag probe bound to a target sequence,  
(e) separating the released reporters electrophoretically, and  
25 (f) detecting separated released reporters, thereby to identify target sequences that hybridized to said probes.

2. The method of claim 1, wherein said extension sequence includes, in a 5'-to-3' direction, a universal primer extension sequence and an e-tag-probe  
30 extension sequence, step (c) includes reacting the extended target sequences with the e-tag probes and with an upstream probe or probes capable of hybridizing to said universal primer extension sequence, and step (d) includes

treating the target sequences with a cleaving agent capable of cleaving said linker only when the extension sequence has both an upstream probe and a tag probe hybridized thereto.

5           3. The method of claim 2, wherein the cleaving agent is a DNA polymerase having 5'-exonuclease activity, and said linker is a nucleotide linked to the 5' end of the oligonucleotide portion through a nuclease cleavable bond.

10           4. The method of claim 2, wherein the cleaving agent is a restriction enzyme, and said linker includes the specific sequence cleaved by said restriction enzyme.

15           5. The method of claim 2, wherein said cleaving agent is a sensitizer capable of generating activated oxygen under conditions of light illumination.

            6. The method of claim 1, wherein the tag probes in a set have the form (D, M<sub>j</sub>)-N-T<sub>j</sub>, which are cleaved to an electrophoretic tag reporter of the form (D, M<sub>j</sub>)-N', where

20           (i) D is a detection group comprising a detectable label or a catalytic group capable of catalyzing a detectable reaction;

            (ii) T<sub>j</sub> is an oligonucleotide target-binding moiety for binding an e-tag probe recognition sequence;

            (iii) N is a linker joined to the 5'-end nucleotide in T<sub>j</sub> through a cleavable bond;

25           (iv) N' is the residue of N remaining after cleavage;

            (v) M<sub>j</sub> is a mobility modifier having a charge/mass ratio that imparts to the corresponding electrophoretic tag, an electrophoretic mobility that is unique to a given extension sequence, and

30           (vi) (D, M<sub>j</sub>)- includes both D - M<sub>j</sub> - and M<sub>j</sub> - D -.

            7. The method of claim 6, wherein N is a nucleotide, and each e-tag probe target binding moiety contains at least one modification selected from a

nuclease-resistant bond joining at least the two 5'-end nucleotides of the target binding moiety, or a capture ligand contained on the 5'-end nucleotide of the target binding moiety and capable of binding specifically to a capture agent.

5           8. The method of claim 6, wherein N is a linkage cleavable by singlet oxygen.

9. The method of claim 1, wherein the e-tag probes in a set have the form:

10 (D, M<sub>j</sub>)<sub>n</sub>-B-N-T<sub>j</sub>, which are cleaved in step (d) under said selected conditions to a branched structure (D, M<sub>j</sub>)<sub>n</sub>-B-N', and are further cleaved to a plurality of electrophoretic tags of the form (D, M<sub>j</sub>)-B', where

(i) D is a detection group comprising a detectable label or a catalytic group capable of catalyzing a detectable reaction;

15           (ii) T<sub>j</sub> is an oligonucleotide target-binding moiety for binding an e-tag probe recognition sequence;

(iii) B is a branched polymer having n electrophoretic tags probes attached thereto, each through a linkage that is cleavage under cleavage conditions different from said selected conditions employed in step (d);

20           (iv) B' is the residue of B remaining after cleavage of the electrophoretic probes from the branched structure;

(v) N' is the residue of N remaining after cleavage of the branched structure from said probe,

25           (vi) M<sub>j</sub> is a mobility modifier having a charge/mass ratio that imparts to the corresponding electrophoretic tag, an electrophoretic mobility that is unique to a given extension sequence, and

30           (vii) (D, M<sub>j</sub>)- includes both D - M<sub>j</sub> - and M<sub>j</sub> - D -, and wherein said treating step includes treating bound probe under said selected conditions to release branched structures in probes hybridized with target sequences, and further treating the released branched structures under said different cleavage conditions, to release electrophoretic probes from the branched structures.

10. The method of claim 9, wherein each of the target binding moieties contains at least one modification selected from a nuclease-resistant bond joining at least the two 5'-end nucleotides of the target binding moiety, or a capture ligand contained on the 5'-end nucleotide of the target binding moiety and  
5 capable of binding specifically to a capture agent.

11. A kit for detecting each or any of a plurality of known, selected nucleotide target sequences, comprising:

(a) a set of forward oligonucleotide primers, each member of the set  
10 containing (ia) a target-sequence that is complementary to one of the known selected target sequences, and (ib) an extension sequence which is unique to the target sequence of that member,

(b) one or more reverse primers that are complementary to said target sequences, said forward and reverse primers being effective, in the presence  
15 enzyme and nucleotide components of a primer extension reaction, to form amplified, extended target sequences, and

(c) a set of electrophoretic tag (e-tag) probes, each member of the tag probe set having (i) an oligonucleotide portion that is complementary to one of said extension sequences, (ii) an electrophoretic probe having an electrophoretic  
20 mobility that is unique to a given extension sequence, and (iii) a linker joining the oligonucleotide portion and the electrophoretic probe, said linker being cleavable under selected conditions when the oligonucleotide portion of said probe is bound to a complementary target extension sequence.

25 12. The kit of claim 11, which further includes enzymes and nucleotide components of a PCR reaction.

13. The kit of claim 11, wherein said extension sequence includes, in a 5'-to-3' direction, a universal primer extension sequence and an e-tag-probe  
30 extension sequence, wherein said kit further includes one or more upstream primers capable of hybridizing to said universal primer extension sequence.

14. The kit of claim 13, for use with a DNA polymerase having 5'-exonuclease activity to cleave said probe, wherein said probe linker is a nucleotide linked to the 5' end of the oligonucleotide portion through a nuclease cleavable bond.

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15. The kit of claim 13, for use with a sensitizer wherein singlet oxygen is generated by photoactivation of said sensitizer, and said one or more upstream primers include a moiety capable of generating singlet oxygen in the presence of light.

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16. The kit of claim 11, for use with a restriction enzyme wherein said probe linker includes or is the specific sequence cleaved by said restriction enzyme.

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17. The kit of claim 11, wherein the tag probes in a set have the form (D, M<sub>j</sub>)-N-T<sub>j</sub>, which are cleaved to an electrophoretic tag of the form (D, M<sub>j</sub>)-N', where

(i) D is a detection group comprising a detectable label or a catalytic group capable of catalyzing a detectable reaction;

(ii) T<sub>j</sub> is an oligonucleotide target-binding moiety for binding an e-tag probe recognition sequence;

(iii) N is a linker joined to the 5'-end nucleotide in T<sub>j</sub> through a cleavable bond;

(iv) N' is the residue of N remaining in after cleavage;

(v) M<sub>j</sub> is a mobility modifier having a charge/mass ratio that imparts to the corresponding electrophoretic tag, an electrophoretic mobility that is unique to a given extension sequence; and

(vi) (D, M<sub>j</sub>)- includes both D - M<sub>j</sub> - and M<sub>j</sub> - D -.

18. The kit of claim 17, wherein N is a nucleotide and each of the e-tag probe recognition sequence-binding moieties contains at least one modification selected from a nuclease-resistant bond joining at least the two 5'-end nucleotides of the recognition sequence, or a capture ligand contained on the 5-

end nucleotide of the recognition sequence and capable of binding specifically to a capture agent.

19. The kit of claim 11, wherein the tag probes in a set have the form:
- 5 (D, M<sub>j</sub>)<sub>n</sub>-B-N-T<sub>j</sub>, which are cleaved in step (d) under said selected conditions to a branched structure (D, M<sub>j</sub>)<sub>n</sub>-B-N', and are further cleaved to a plurality of electrophoretic tags of the form (D, M<sub>j</sub>)-B', where
- (i) D is a detection group comprising a detectable label or a catalytic group capable of catalyzing a detectable reaction;
  - 10 (ii) T<sub>j</sub> is an oligonucleotide target-binding moiety;
  - (iii) B is a branched polymer having n electrophoretic tags probes attached thereto, each through a linkage that is cleavage under cleavage conditions different from said selected conditions employed in step (d);
  - (iv) B' is the residue of B remaining after cleavage of the electrophoretic
  - 15 probes from the branched structure;
  - (v) N is a linker joined to the 5'-end nucleotide in T<sub>j</sub> through a cleavable bond; and N' is the residue of N remaining after cleavage of the branched structure from said probe;
  - (vi) M<sub>j</sub> is a mobility modifier having a charge/mass ratio that imparts to the
  - 20 corresponding electrophoretic tag, an electrophoretic mobility that is unique to a given extension sequence; and
  - (vii) (D, M<sub>j</sub>)- includes both D - M<sub>j</sub> - and M<sub>j</sub> - D -.

20. The kit of claim 19, wherein N is a nucleotide and each e-tag probe
- 25 target binding moiety contains at least one modification selected from a nuclease-resistant bond joining at least the 5'-end nucleotides of the recognition sequence, or a capture ligand contained on the 5'-end nucleotide of the target binding moiety and capable of binding specifically to a capture agent.

- 30 21. The kit of claim 20, further comprising a cleaving moiety selected from the group consisting of a 5' exonuclease, a restriction enzyme, an RNase, a protease and an esterase.